

Appl. No.: 10/651,499  
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Amendments to the Claims:

1. (Original) A crystallization cassette comprising:  
a support member having a top, middle, and bottom portion;  
a housing member joined to the top portion;  
a stabilizing member joined to the middle portion having a plurality of capillary passageways;  
a precipitating reservoir member joined to the bottom portion, whereby the capillary tubes are in fluid communication with the precipitating reservoir; and  
a plurality of capillary tubes each having a proximal and distal end, wherein the capillary tubes' proximal ends are each joined to the housing member, and wherein each capillary tube extends downwardly from the housing member through a capillary passageway.
2. (Original) A crystallization cassette according to Claim 1, wherein the precipitating reservoir member includes:  
a surface facing the distal ends of the capillary tubes; and  
a plurality of cavities on the surface, wherein each cavity respectively corresponds to a capillary tube.
3. (Original) A crystallization cassette according to Claim 1, wherein the capillary tubes are suitable for X-ray diffraction.
4. (Original) A crystallization cassette according to Claim 1, wherein the capillary tubes are selected from the group consisting of quartz, acrylic poly(methyl methacrylate), polystyrene, mylar polycarbonate, CR 39, copolymers of styrene and poly(methyl methacrylate), and their derivatives, and combinations thereof.
5. (Original) A crystallization cassette according to Claim 4, wherein the capillary tubes are quartz.
6. (Original) A crystallization cassette according to Claim 1, wherein the capillary tubes have a diameter from about 0.05 mm to 1 mm.

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7. (Original) A crystallization cassette according to Claim 1, wherein the capillary tubes have a diameter about 0.3 mm or less.
8. (Original) A crystallization cassette according to Claim 1, wherein the support member is a shaft.
9. (Original) A crystallization cassette according to Claim 2, wherein the precipitating reservoir member further includes a pierceable layer on the surface.
10. (Original) The crystallization cassette according to Claim 9, wherein the pierceable layer is selected from the group consisting of latex, wax, plastic plug, agarose, and fracture ease.
11. (Original) The crystallization cassette according to Claim 2, wherein the plurality of cavities each have a volume from about 5 to 50  $\mu\text{L}$ .
12. (Original) A crystallization cassette comprising:
  - a shaft having a top, middle, and bottom portion;
  - an upper member joined to the top portion of the shaft;
  - a middle member proximate to the middle portion of the shaft including:
    - a first channel for receipt of the shaft; and
    - a plurality of capillary passageways that are substantially parallel to the shaft and extend longitudinally through the middle member;
  - a plurality of capillary tubes each having a proximal and distal end, wherein the capillary tubes extend downwardly from the upper member, and wherein each capillary tube extends downwardly through a capillary passageways; and
  - a lower member proximate to the lower portion of the shaft having
    - a first surface having a plurality of cavities located on the surface, wherein each cavity is in alignment and corresponds to a capillary tube,
    - a second surface, opposite the first surface, and
    - a second channel for receipt of the shaft, whereby the capillary tubes are in fluid communication with the lower member.

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13. (Original) The crystallization cassette according to Claim 12, wherein the shaft has a vertical axis extending from the lower member to the upper member and a keyway extending longitudinally along the axis.

14. (Original) The crystallization cassette according to Claim 13, wherein the lower member has a key disposed in the second channel such that the key is adapted to slide along the keyway.

15. (Original) The crystallization cassette according to Claim 13, wherein the middle member has a key disposed in the first channel such that the key is adapted to slide along the keyway.

16. (Original) The crystallization cassette according to Claim 12, wherein the upper member includes a plurality of independently pivotable capillary housing sections, wherein each pivotable housing section is joined to the proximal end of a capillary tube and has a hinge disposed proximate to the shaft, and wherein the pivotable housing section is adapted to pivot upwardly such that the capillary tube is extended outwardly.

17. (Original) The crystallization cassette according to Claim 16, wherein the outwardly extended capillary is substantially perpendicular to the vertical axis of the shaft.

18. (Original) The crystallization cassette according to Claim 12, wherein the middle member has an outer edge that is parallel to the vertical axis, the outer edge having a plurality of channels that extend laterally through the middle member from the outer edge to the plurality of passageways such that a capillary tube is reversibly insertable through the channel into the passageway.

19. (Original) The crystallization cassette according to Claim 12, wherein the capillary tube has a ferrel disposed proximate to the distal end.

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20. (Original) The ferrel according to Claim 19, having a substantially circular body with a lower and upper portion, and a base rim disposed at the lower portion having a diameter larger than the body.

21. (Original) The ferrel according to Claim 20, having a beveled edge disposed at the upper portion.

22. (Original) The crystallization cassette according to Claim 19, wherein the diameter of the passageways are the same or larger than the diameter of the body and smaller than the diameter of the rim such that as the middle disc is slid downwardly along the shaft the bodies of the ferrels will slide into the passageways.

23. (Original) The crystallization cassette according to Claim 12, wherein the first channel is disposed at the center of the middle member.

24. (Original) The crystallization cassette according to Claim 12, having a positioning lock for holding the middle member stationary relative to the shaft including:  
a positioning channel extending laterally through the middle member from the outer edge to the first channel;  
a positioning recess on the shaft that is perpendicular to the vertical axis and is aligned with the positioning channel; and  
a positioning pin that is reversibly insertable into the positioning channel such that as the middle member is slidably moved along the shaft the positioning pin can be inserted through the positioning channel into the positioning recess to thereby lock the middle member's position.

25. (Original) The crystallization cassette according to Claim 12, wherein the lower member has a pierceable layer disposed on the first surface.

26. (Original) The crystallization cassette according to Claim 25, wherein the pierceable layer is selected from the group consisting of latex, wax, plastic plugs, agarose, and fracture ease.

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27. (Original) The crystallization cassette according to Claim 12, wherein the plurality of cavities each have a volume from about 5 to 50  $\mu\text{L}$ .

28. (Original) The crystallization cassette according to Claim 12, wherein the lower member has a first depth control member for controlling the depth to which the capillary tubes are inserted into the cavities.

29. (Original) The crystallization cassette according to Claim 28, wherein the first depth control member is a stud extending outwardly from the first surface in a direction that is substantially parallel to the vertical axis of the shaft.

30. (Original) The crystallization cassette according to Claim 28, wherein the lower member's orientation to the shaft is inverted so that the second surface is facing the middle member.

31. (Original) The crystallization cassette according to Claim 28, wherein the lower member has a second depth controlled member on the second surface for controlling the distance between the distal ends of the capillaries and the second surface.

32. (Original) The crystallization cassette according to Claim 31, wherein the depth control member is a second stud having a height that is greater than the height of the first depth control member, and wherein the second stud extends outwardly from the second surface in a direction that is substantially parallel to the vertical axis of the shaft.

33. (Original) The crystallization cassette according to Claim 12, wherein the second channel is disposed at the center of the lower member.

34. (Original) The crystallization cassette according to Claim 12, wherein the capillary tubes are suitable for X-ray diffraction.

35. (Original) The crystallization cassette according to Claim 12, wherein the capillary tubes are selected from the group consisting of quartz, acrylic poly(methyl

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methacrylate), polystyrene, mylar polycarbonate, CR 39, copolymers of styrene and poly(methyl methacrylate), and their derivatives and combinations thereof.

36. (Original) The crystallization cassette according to Claim 12, wherein the capillary tubes are quartz.

37. (Original) The crystallization cassette according to Claim 12, wherein the capillary tubes diameters are from about 0.05 mm to 1 mm.

38. (Original) The crystallization cassette according to Claim 12, wherein the capillary tubes diameters are about 0.3 mm or less.

39. (Original) The crystallization cassette according to Claim 12, wherein the upper, middle, and lower members are disc shaped.

40. (Original) The crystallization cassette according to Claim 12, wherein the cassette has 12 capillary tubes.

41. (Original) The crystallization cassette according to Claim 12, wherein the overall shape of the cassette is cylindrical.

42. (Original) The crystallization cassette according to Claim 12, wherein the shaft, upper member, and lower member are comprised of an amorphous non-refractive plastic.

43. (Original) The crystallization cassette according to Claim 12, wherein the cassette is made from a material selected from the group consisting of quartz, acrylic poly(methyl methacrylate), polystyrene, mylar polycarbonate, CR 39, copolymers of styrene and poly(methyl methacrylate), and their derivatives and combinations thereof.

44. (Original) A crystallization cassette according to Claim 12, wherein the plurality of cavities each respectively have a lower portion and an upper portion.

45. (Original) A crystallization cassette according to Claim 44, having a pre-loaded lower member comprising:

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a capillary sealant disposed in each cavities' lower portion;  
a precipitating solution, cryoprotectant solution, and a high X-ray scattering atom component disposed in the cavities upper portion; and  
a pierceable member disposed on the first surface such that the cavities are sealed.

46. (Original) A crystallization cassette according to Claim 45, wherein the cavity sealant is selected from the group consisting of wax and clay.

47. (Original) A crystallization cassette according to Claim 45, wherein the precipitating solution is selected from the group consisting of salts and alcohols.

48. (Original) A crystallization cassette according to Claim 45, wherein the cryoprotectant solution is selected from the group consisting of primary alcohols, glycerol, polyethylene glycol, methylpentanediol, and derivatives thereof.

49. (Original) A crystallization cassette comprising:  
a shaft having a top, middle, and bottom portion;  
a capillary housing member joined to the top portion of the shaft;  
a capillary stabilizing member joined to the middle portion of the shaft having a first channel that is adapted for slidably receiving the shaft, and a plurality of passageways that are parallel to the vertical axis of the shaft and extend longitudinally through the stabilizing member;  
a plurality of capillary tubes having a proximal and distal end, wherein the capillary tubes extend downwardly from the housing member, and wherein each capillary tube extends downwardly through one of the passageways; and  
a precipitation reservoir member joined to the lower portion of the shaft having a surface facing the stabilizing member,  
a plurality of cavities located on the surface, wherein each cavity is in alignment and corresponds to a capillary tube, and  
a second channel that is adapted for slidably receiving the shaft.

50. (Original) A method of growing biological crystals comprising the steps of:

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- a. providing a crystallization cassette as described in Claim 45 having a pre-loaded lower member;
- b. depositing a protein solution on the pierceable layer above each cavity;
- c. repositioning the lower member along the shaft until the distal ends of the capillary tubes contact the protein solutions;
- d. diffusing the protein solutions into the capillary tubes;
- e. piercing the pierceable layer with the distal end of the capillary tubes by repositioning the lower member towards the middle member;
- f. contacting the distal ends of the capillary tubes with the precipitating solution, cryoprotectant solution, and scattering atom component;
- g. diffusing the precipitating solution, cryoprotectant solution, and scattering atom component into the capillary tubes;
- h. growing biological crystals in the capillary tubes;
- i. sealing the distal ends of the capillary tubes with the capillary sealant; and
- j. selecting crystals for X-ray diffraction.

51. (Original) The method for growing biological crystals according to Claim 50, wherein the step of providing a pre-loaded loaded member further comprises the steps of:

- a. inserting a capillary sealant within the cavities;
- b. inserting precipitating, cryoprotectant, and scattering atom solutions into each of the cavities; and
- c. sealing the cavities with a pierceable layer;

52. (Original) The method for growing biological crystals according to Claim 50, further comprising the step of analyzing the crystals *in situ*.

53. (Original) The method for growing biological crystals according to Claim 52, wherein the step of analyzing crystals *in situ* further comprises the steps of:

- a. mounting the cassette in an X-ray diffractometer on a motorized adaptor for rotating the cassette;



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- b. extending a capillary tube outwardly from the cassette;
- c. applying a stream of cryogenic gas to the capillary tube;
- d. applying an X-ray beam to a desired location on the extended capillary tube;
- e. rotating the capillary in the X-ray beam; and
- f. collecting x-ray data.

54. (Original) The method for growing biological crystals according to Claim 50, wherein the biological crystal is selected from the group consisting of proteins, nucleic acids, and viruses.

55. (Original) The method for growing biological crystals according to Claim 50, wherein the volume of protein solution and precipitating solution are about a 1:1 ratio.

56. (Original) A cassette positioning system for rotating a crystallization cassette about an x-axis and y-axis comprising:

- an X-ray source;
- a turntable for rotating the cassette about the y-axis of rotation;
- an eccentricity correction stage disposed below the turntable;
- a capillary scan stage disposed under the eccentricity stage; and
- a cassette rotation stage for rotating a the cassette about the x-axis.

57. (New) A method of growing and analyzing macromolecules comprising:

- providing a plurality of capillary tubes each having a first and second end;
- contacting the second end of each capillary tube to a solution having a solvated macromolecule therein;
- diffusing the solution into the capillary tubes;
- inserting the second end of each capillary tube into a corresponding cavity having one or more precipitating agents therein;
- allowing the one or more precipitating agents to counter-diffuse against the solution in each capillary tube;
- allowing macromolecule crystals to grow in the capillary tubes; and

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analyzing the crystals *in situ* via x-ray diffractometry.

58. (New) The method according to Claim 57, further comprising sealing the second end of each capillary tube.

59. (New) The method according to Claim 57, wherein the macromolecule comprises a protein.

60. (New) The method according to Claim 57, wherein the precipitating agent further comprises a cryoprotectant solution, scattering atom component, or combination thereof.

61. (New) The method according to Claim 57, further comprising disposing a pierceable layer between the solution and each corresponding cavity, and wherein the solution is in the form of a droplet.

62. (New) The method according to Claim 61, wherein the step of inserting the second end of each capillary tube into a corresponding cavity further comprises piercing the pierceable layer with the second end of each capillary tube.